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THE COEFFICIENT OF MUTATION IN *OENOTHERA*  
BIENNIS L.

HUGO DEVRIES

The significance of the discovery of the mutability of *Oenothera Lamarckiana*, *O. biennis*, and allied forms is a double one. In the first place, it provides us with material for experimental investigations into the laws which govern the origin of living forms by means of the production of new characters and of the loss of existing ones. The knowledge of such laws must become of the highest practical value as soon as the evidently limited possibilities of producing new forms through the recombination of characters by means of crossing becomes exhausted. This conclusion seems especially well founded, since the old conception of improving agricultural races after the principle of slow and continued selections has now generally been abandoned and replaced by the direct selection of elementary types out of the mixtures which constitute the so-called agricultural races and varieties.

The appearance of really new characters seems to be a very rare phenomenon in nature, and a case in which such changes regularly occur in one or more per cent of all the individuals affords material for experiments, the results of which may be expected to apply to a large series of other species also, including, probably, an important number of agricultural crops.

In the second place, the mutability of the evening primroses has a distinct bearing upon the theory of mutation, or of the origin of all living species from one another by sudden leaps instead of

by slow and almost invisible changes as was assumed by DARWIN. The theory itself does not, of course, depend on this or other single instances; it is founded upon general considerations taken from almost all branches of biological and paleontological research, as I have often pointed out.<sup>1</sup>

One of the main arguments is the statement that adaptations cannot, as a rule, have been produced by slow improvements, and that quite a large number of differentiations in organization, if not almost all the really important ones among them, are not adaptations at all.

Apart from our poetical admiration of nature, we have no other way of judging the reality and efficiency of supposed adaptations than by their effects in the struggle for life. Species which are distributed over large countries and occur in thousands of individuals are evidently well fitted for their life conditions. Newly introduced forms, which are spreading with astonishing rapidity and gaining a large territory often in the lapse of a few years, thereby show the highest degree of adaptation to their new environment. But a showy differentiation may be followed by a wide distribution, as in the case of *Drosera*, or limit the species to a relatively very small area, as in *Dionaea*.

Of late J. C. WILLIS has brought forward the most conclusive evidence against the theory of natural selection and in favor of an origin of species by mutation.<sup>2</sup> He bases some of his arguments upon his observations of the endemic species of Ceylon, such as are found in *Coleus*, *Acrotrema*, and other genera. If these endemics had evolved according to the law of natural selection, in consequence of a gradually increasing adaptation to their local environment, it would follow that they must now be better adapted than their parent types, conquer these in the struggle for life, and become quite common, while the old forms would tend to disappear. As a matter of fact, however, their behavior is quite the contrary.

<sup>1</sup> DEVRIES, HUGO, The mutation theory. 2 vols. 1909-1910; Species and varieties, their origin by mutation, 2d ed., 1906; Die Mutationen in der Erblichkeitslehre. pp. 42. Berlin. 1912; The principles of the theory of mutation. Science 40:77-84. 1914.

<sup>2</sup> WILLIS, J. C., Some evidence against the theory of the origin of species by natural selection of infinitesimal variations, and in favor of origin by mutation. Ann. Roy. Bot. Gard. Peradeniya 4:1-15. 1907.

The endemics are rare, often strictly local, and grow in the midst of a luxuriant vegetation of their widely spread and thriving ancestors. It is hardly necessary to point out that this conclusion holds good not only for Ceylon, but for the origin of endemic and local species in general.

WILLIS has also called attention to the Podostemaceae and the allied group Tristichaceae. They show one of the most interesting illustrations of a very rich differentiation without the least indication of a relation to their environment. A very great uniformity in the conditions of life is combined with a most remarkable variety in their morphological structure. In the Podostemaceae the flowers are anemophilous, terminal, and erect, but combine with these characters of low organization the highest degrees of dorsiventrality and of differentiation, and this without any reference to advantages or disadvantages to be derived from them in their functions. Numerous points of similar significance in the structure of the vegetative and reproductive organs are pointed out by the author. Moreover, the genera *Tristicha* and *Podostemon*, which are widely distributed, are comparatively little modified from the earlier types of the orders, while the highly specialized forms are at the same time the rarest, exactly as in the case of the endemics of Ceylon.<sup>3</sup>

In the group of the evening primroses the same principles prevail. Their struggle for existence is limited by the difficulties, which they have in producing roots. Cuttings almost never succeed in rooting, with the exception of the lateral rosettes at the base of the stem. Artificial transplanting becomes difficult as soon as the main root increases in size. In the field only a small percentage of the seeds germinate and thrive, and this only under special conditions. They want a stirred up soil and do not like to grow between other plants. These characters are common to all the forms which I have had an opportunity of studying in their native habitats. On the other hand, the numerous small specific differentiations, such as the form of the leaves, the branching of the stem, or the structure of the flowers and fruits, do not show

<sup>3</sup> WILLIS, J. C., On the lack of adaptation in the Tristichaceae and Podostemaceae. Proc. Roy. Soc. 8:532-550. 1914.

the least relation to their environments. Even the preference for an annual or a biennial behavior, which might seem to be a direct adaptation, does not exhibit any reference to the actual life conditions. The conception of natural selection and of the accumulation of small variations on account of their utility cannot explain the specific and generic differences in this group.

Therefore it seems unavoidable to assume that specific differentiation in the genus *Oenothera* has been produced and is still being produced by small steps, each of which evolved a character at once to its full development, without any reference to the struggle for life. That, besides this process, from time to time new combinations of characters by means of crosses may have given rise to constant hybrid strains, which we have as yet no means of distinguishing from pure species, cannot of course be doubted.

Now, *Oenothera Lamarckiana*, *O. biennis*, and some allied forms are seen to be still in a condition of making, from time to time, such small steps. They are doing this in their natural habitats as well as in experimental cultures, and the variations produced show no relation to the external conditions of their environment or to the method of their culture. On this ground, the claim seems justified that the mutations, directly observed in the primroses, are similar to those which have produced in nature the specific differences and the differentiating characters in this group. If this is conceded, it follows that the analogous processes in other genera, and even in the origin of the larger systematic groups, must be viewed in the same way. This claim, however, has not escaped serious objections.

The main line of these attacks is based upon the vague and double assumption that *O. Lamarckiana* might be a hybrid, and that its hybrid origin might account for its present mutability. These two assumptions are evidently independent of one another and would have to be proven separately. So far as I know, no attempts have been made as yet to prove the second assumption, and no hybrid races have been produced which, from this cause, give rise to phenomena exactly duplicating the mutations of the primroses. And it is evident that so long as such an analogy is only an ardent wish of the critics, the question whether the mutating

primroses are of pure or of hybrid origin is not of paramount importance for the appreciation of the fact of their mutability.

The first attacks on the gametic purity of the mutating forms have been directed only against *O. Lamarckiana*, and at the present time the most prominent adherents of this opinion are DAVIS and RENNER. They try to give proof of a separate hybrid nature for this species on considerations which do not apply to *O. biennis* and the other mutating forms, and concede for these latter a pure origin.<sup>4</sup>

DAVIS based his arguments upon a historical research concerning the origin of *O. Lamarckiana*, and upon his attempts to duplicate this form by crossing others which he assumed to be of pure line.<sup>5</sup> A specimen collected by MICHAUX in the eastern part of the United States, about a century ago, and studied by L. BLARINGHEM, proves our plant to have been a component of the flora of this country, whence LAMARCK obtained the authentic specimen for his original description.<sup>6</sup>

RENNER studied the empty seeds of *O. Lamarckiana*, which constitute over one-half of the whole crop. He brings this phenomenon in connection with the ability of this species to produce twin hybrids, *laeta* and *velutina*, in certain crosses with older types, and assumes that the sexual cells are one-half potential *laeta*, and the other half potential *velutina*. In the normal fertilization of *O. Lamarckiana* this would produce  $\frac{1}{4}$  *laeta*,  $\frac{1}{4}$  *velutina*, and  $\frac{1}{2}$  of the hybrid combination *laeta* × *velutina*. He further assumes that in pure condition both the *laeta* and *velutina* qualities are incompatible with normal development, and that the germs which bear them are doomed to die at an early stage, thereby leaving their seeds empty. Only the combination *laeta* × *velutina* would be fit for further growth, and if we assume that this shows the marks of *O. Lamarckiana*, the constancy of this form would not be in

<sup>4</sup> DAVIS, B. M., Mutations in *Oenothera biennis* L. Amer. Nat. 47:116. 1913; and Parallel mutations in *Oenothera biennis*. Ibid. 48: 499-501. 1914.

<sup>5</sup> ———, Some hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. Amer. Nat. 45:193-233. 1911.

<sup>6</sup> BLARINGHEM, L., L'*Oenothera Lamarckiana* Ser. et les *Oenothères* de la forêt de Fontainebleau. Travaux de biologie végétale, dédiés à GASTON BONNIER. Rev. Gén. Bot. 25<sup>2</sup>:35-50. 1914; see also my article in BOT. GAZ. 57:345-360. pls. 17-19. 1914.

contradiction with the other hypotheses. If we accept these views, all reasons for supposing a correlation between the splitting phenomenon and the mutability would lose their value, and this latter process would come much nearer to the corresponding changes in *O. biennis* and allied species. The hypothesis, although resting on too large a number of suppositions, would in some sense be a support for the theory of mutation, since it is evidently impossible that these presumed qualities, which are incompatible with life, could have evolved slowly on the ground of their utility in the struggle for existence. Moreover, the hypothesis has no direct bearing on the observed phenomena of mutation, and the fact that in *O. biennis* such empty seeds are wholly or almost wholly absent proves beyond doubt that mutability may be independent of them. Thus the hypothesis of RENNER emphasizes the importance of a study of the mutation phenomena in *O. biennis*, in contradistinction to those in *O. Lamarckiana*, at least for the present, until facts are available to appreciate the correctness of his views.

Obviously the hypothesis that *O. Lamarckiana* might be a hybrid, whilst *O. biennis* is not, can in no way account for the phenomena of mutation which are common to both of these species. For this reason it seems important to describe the degree of mutability as it has been observed, up to this time, in *O. biennis*, which is, next to *O. Lamarckiana*, the most suitable species for this kind of research. The mutations in the other forms seem to be far more rare, and therefore require many more thousands of individuals for a statistical study or for experiments upon their causes.

Besides the assumption that *O. Lamarckiana* might be a hybrid, some authors have recently pointed out that hybridism may be one of the chief ways in which species are produced in nature, especially in the larger or so called polymorphous genera. LINNAEUS was the first to propose this hypothesis, at the time when the number of discovered forms was growing so fast as to make it almost impossible to assume a separate creation for every one of them. I have not the least doubt that LINNAEUS and his followers were right in this point, and that many wild species have been produced by the sexual combination of the characters of their allies. How great a rôle this kind of hybridization or of the recombination of char-

acters has played in the production of species in nature is a question which it is impossible to answer at the present time. There is no doubt that numerous hybrids are continually produced in nature, but almost all of them disappear after a relatively short period of existence. Even in such genera as *Cirsium* and *Salix*, which are known to be rich in hybrids, our knowledge concerning the propagation of hybrid strains is very small.<sup>7</sup> It is quite possible that some as yet undiscovered principle of purification (*Selbstreinigung der Arten*) prevails on a large scale, and if this should be so, we must expect hybrid races to be rather rare in the field.

FOCKE has published a list of forms which have been duplicated by means of artificial crosses,<sup>8</sup> and quite a number of later instances have been added to this list, the latest of them being the reconstruction of *O. biennis leptomeres* out of *O. biennis* L. and *O. atrovirens* Bartlett (*O. cruciata* of my *Gruppenweise Artbildung*), by means of the expulsion of the undesirable characters in double reciprocal crosses.<sup>9</sup> But all such facts point rather to a relative rarity of hybrid races in nature, outside of the small number of well known polymorphic genera.

GATES assumes that crosses between species or between elementary species often occur in nature among allogamous or open-flowered forms.<sup>10</sup> But, according to my own experience, even in such cases hybrids are rare in the wild state, and hybrid races must be much rarer still. The slightest degree of weakening of the individual vigor will doom such hybrids to extermination, even as most of the occasional white flower mutations in nature disappear sooner or later, without starting a permanent variety.

In order to save the hypothesis of hybridism as a cause of the mutable condition of the evening primroses, different authors have

<sup>7</sup> For the hybrids of *Cirsium* see C. NÄGELI, *Dispositio specierum generis Cirsii tam genuinarum quam hybridarum*, in G. D. J. KOCH, *Synopsis Florae Germanicae et Helveticae*, pp. 743-760. 1857; and for the willows see MAX WICHURA, *Die Bastardbefruchtung im Pflanzenreich, erläutert an den Bastarden der Weiden*. Breslau. pp. 95, mit zwei Tafeln. 1865.

<sup>8</sup> FOCKE, W., *Die Pflanzenmischlinge*. 465-468. 1881.

<sup>9</sup> *Gruppenweise Artbildung*. Berlin. 311-312. 1913.

<sup>10</sup> GATES, R. R., *Mutation in Oenothera*. *Amer. Nat.* 45:577-606. 1911; see pp. 578-579.

proposed different auxiliary suppositions. And since the possibility is acknowledged that mutability may be far more widely spread within this group than we now know, such suppositions must not be of a limited nature, but applicable to large divisions of the vegetable kingdom. KEARNEY, in studying the mutations of the Egyptian cotton, comes to the conclusion that these and other mutations might be the result of crosses between remote ancestors, but that these crosses have left no other traces in their descendants than "the disturbance of germinal equilibrium, which manifests itself in the production of mutants."<sup>11</sup> It is not very clear how this supposition is to bring the problem nearer to its solution.

In a recent article in this journal,<sup>12</sup> JEFFREY takes an opposite position. He assumes that the ancestral crosses have left another visible trace in their descendants, which is the partial sterility of their sexual cells. It is a well known fact that many hybrids have partially sterile pollen, while acknowledged species have, as a rule, only fertile pollen grains. JEFFREY assumes this rule to be without exceptions, but does not adduce any arguments in favor of this hypothesis. It is difficult to judge the value of an argument so long as the facts upon which it rests have not been submitted to criticism. But I might suggest that it seems rather hard to reconcile this view with the fact that in angiosperms three of the four megaspores are usually sterile, while only one produces an embryo sac. Are we to deduce from this fact, in connection with JEFFREY'S hypothesis, that all angiosperms are hybrids, at least on the maternal side?

Numerous special arguments could be adduced. It may suffice, however, to point out the genus *Carex*, in some of the best species of which the pollen is in the same condition, three of the grains of each tetrad being sterile and only one fertile.<sup>13</sup> Every single grain of the ripe pollen is a tetrad, showing the very reduced rudimentary remnants of three of its cells as a flattened investment of the fertile one.

<sup>11</sup> KEARNEY, T. H., Mutation in Egyptian cotton. Jour. Agric. Research 2:287-302. 1914.

<sup>12</sup> JEFFREY, E. C., Spore conditions in hybrids and the mutation hypothesis of DEVRIES. BOT. GAZ. 58:322-336. 1914.

<sup>13</sup> JUEL, H. O., Die Entwicklung der Pollenkörner bei *Carex*. Jahrb. Wiss. Bot. 35:649-656. 1900.



In the article just quoted, no parallelism has been attempted between the presence of sterile pollen grains and the already numerous published instances of mutations outside of the group of the evening primroses. Let us take for instance *Capsella Bursa-pastoris*, which has produced *C. Heegeri* and *C. Viguieri*.<sup>14</sup> Its pollen is devoid of sterile grains. Here we have a clear case of partial sterility not being the cause of mutability. On JEFFREY'S principle we must acknowledge *C. Bursa-pastoris* as a good species of undoubted gametic purity, and therefore it is evident that even the purest species may be in a mutable condition. From this we infer that mutability in itself does not justify the supposition of a hybrid origin, and that attacks on the gametic purity of the evening primroses have no real support on this side of the question. I have cultivated both *C. Heegeri* and *C. Viguieri* in my experiment garden; the first of them has globular and the other four-winged capsules. Both are historically known to have arisen suddenly from the parent stock, and come true to seed.

JEFFREY lays stress mainly on the fact that partially sterile pollen is a widespread phenomenon among the allies of the evening primroses. Whether it runs parallel to their mutability has not been investigated, and as a matter of fact it does not seem to be much more highly developed in *O. Lamarckiana* and *O. biennis* than in the other members of the group.

The question of the partial sterility of the Onagraceae has been most thoroughly dealt with by GEERTS.<sup>15</sup> He sums up his results as follows: The genera *Jussieua*, *Zauschneria*, *Epilobium*, *Boisduvallia*, and *Lopezia* are wholly fertile; they show neither rudimentary ovules nor sterile pollen grains. Only in *Epilobium* and *Boisduvallia* some rare pollen tetrads may sometimes miscarry. In the genera *Clarkia*, *Eucharidium*, *Godetia*, and *Gaura* all the ovules are fertile, but among the pollen grains about 30 per cent

<sup>14</sup> SOLMS-LAUBACH, H., *Capsella Heegeri* Solms, eine neu entstandene Form der deutschen Flora. Bot. Zeit. 10:167-190. pl. 7. 1900.

BLARINGHEM, L., Fleurs prolifères du *Cardamine* des prés. Bull. Soc. Bot. France 60:304-311. 1913; and Les transformations brusques des êtres vivants. Bibl. Phil. Sci. Paris. 1911 (see pp. 119-147).

<sup>15</sup> GEERTS, J. M., Beiträge zur Kenntnis der Cytologie und der partiellen Sterilität von *Oenothera Lamarckiana*, Amsterdam. pp. 114, mit 24 Tafeln. 1901; see p. 93.

are sterile. *Kneiffia*, *Xylopleurum*, and *Lavauxia* have some rudimentary ovules as well as sterile pollen grains (10-50 per cent). In the genus *Oenothera*, with the subgenera *Onagra*, *Euoenothera*, and *Anogra*, the percentage of sterility is about 50 per cent in the ovary as well as in the anthers. In the first group about 40 species were studied, in the second 30, in the third 10, and in the last 40, making together about 120 species. If in the last three groups some species were pure, and devoid of sterile sexual cells, they would no doubt have been discovered, and the supposition that the remainder might be considered as their hybrids would have found support. But this was not the case, and if we wish to ascribe the presence of all these sterile sexual cells to ancestral crosses, the crosses must be supposed to have taken place, or at least to have begun, among the ancestors of the whole family, with the exception of the *Lopezieae*, the *Jussieueae*, and the *Epilobieae*. It seems hard to have to suppose that the whole pedigree of the *Xylopleurinae*, the *Clarkiinae*, and the *Oenotherinae* should have had to go through the development of partial sterility in order to produce the present mutability of *Oenothera Lamarckiana* and half a dozen or perhaps even a dozen of its nearest allies.

The second main supposition, namely that hybridism might be a cause of mutability, is dealt with by JEFFREY in a particular way. He assumes "that there is every reason to suppose that it has been an agency of great importance in *multiplying* species, although it is logically inconceivable in the present state of our biological knowledge that it could have presided at their origin." The first of these two alternatives represents, so far as I can see, a conviction which is at least very widely spread among biologists ever since the time of LINNAEUS. It by no means contradicts the theory of natural selection, nor that of mutation, nor any other evolutionary principle. It has no obvious reference to the phenomena observed in the evening primroses, since with them the production of new forms takes place in pure lines of a species which has come down to us unchanged during at least a century, since the time MICHAUX discovered it in the United States and sent it to Europe.<sup>16</sup> At least there is no direct recombination of characters

<sup>16</sup> The probable origin of *Oenothera Lamarckiana*. BOT. GAZ. 57:345-360. 1914; see *pl.* 19.

by actual crosses between different elementary types, such as we ordinarily suppose to occur in polymorphic groups in nature.

The other alternative, that it is logically inconceivable that hybridism could have presided at the *origin* of new species, coincides exactly with the current conception of the mutability in the evening primroses. New forms originate through the evolution of new characters, as in *O. gigas* and *O. rubricalyx*;<sup>17</sup> or through the loss of existing ones, as in *O. nanella* and *O. rubrinervis*; or by means of the appearance of qualities, which were probably latent in the parent race, as in *O. lata* and *O. scintillans*.<sup>18</sup> These cases are evidently not recombinations of existing characters. If it is conceded that the hypothesis of a hybrid origin does not apply to them, it is obviously unimportant for the theory whether or not, besides them, there are other instances which may be considered as hybrid recombinations. *O. semigigas*, which is a hybrid between a normal and a mutated sexual cell, has never been considered as an argument against the mutation theory.

In cultures of chrysomelid beetles, W. L. TOWER has observed hereditary changes which run almost parallel to the mutations of *O. Lamarckiana*. He started from crosses between *Leptinotarsa decemlineata*, *L. multitaeniata*, and *L. oblongata*, and obtained constant races. When given proper treatment by changing their environic factors, these races could be made to break up, and they did so in a manner at least partially analogous to that of the evening primroses.<sup>19</sup>

It is obvious that the fact that mutations may be artificially induced in hybrid strains does not contradict the contention that they may arise in pure strains also. But from the experiments of TOWER it seems that some hybrid strains at least are more liable to show the phenomenon.

<sup>17</sup> *O. gigas* is considered to be a progressive mutant on account of its double number of chromosomes and its special behavior in crosses. *O. rubricalyx*, which arose in the cultures of GATES from *rubrinervis*, and which I cultivated this summer from seeds kindly supplied by him, is perhaps the most beautiful among all the mutants of *O. Lamarckiana*. Its red color is something quite new in the group. It behaves as a Mendelian dominant in crosses with its parent species and is therefore obviously of a progressive nature; see GATES, R. R., Amer. Nat. 45:600. 1911.

<sup>18</sup> See Gruppenweise Artbildung. Berlin. pp. 244-260. 1913.

<sup>19</sup> TOWER, W. L., Evolution of the chrysomelid beetles. Carnegie Institution of Washington Yearbook no. 12:68-71. pl. 3. 1913.

Let us now consider the production of new forms analogous to the mutations of *O. Lamarckiana* observed in allied species. The theoretical significance of these facts lies in the proof that any hypothesis to explain such phenomena on the ground of qualities which are special to LAMARCK'S evening primrose is to be considered as wholly inadequate.

The first instance of mutability shown by another species than *O. Lamarckiana* was the production of a dwarf by *O. biennis cruciata*, a form which is now to be described as *O. biennis* var. *leptomeres* Bartl. This form was first discovered in 1900 by my son ERNST DE VRIES in the sand dunes near Santpoort in Holland, where a single specimen of *O. biennis* bore linear petals, while all the surrounding individuals were normal *O. biennis* L. It had evidently arisen there by mutation.<sup>20</sup> From it a constant strain has been derived, which is still in cultivation.<sup>21</sup> Among about 600 plants of this variety a single dwarf arose in 1903.<sup>22</sup> It had all the marks of *O. biennis* L. combined with the stature of a dwarf and the linear petals of the parent form.

Shortly afterward STOMPS discovered, in his cultures of hybrids between this *cruciata* variety and the original species, another dwarf and, moreover, a new mutant type, *O. biennis semigigas*.<sup>23</sup> Both arose from guarded seeds without any intermediate steps, in the same way that the mutants of *O. Lamarckiana* are known to arise. They had cordate petals, the dwarf having in other respects the same characters as the dwarf of *O. biennis leptomeres*, and the *semigigas* having 21 chromosomes in its nuclei. STOMPS was the first to lay stress on these facts as a proof that mutability is not limited to *O. Lamarckiana*, and that, even if this latter species should have to be considered as a hybrid, mutability cannot be explained as a result of such a condition, since there is not the least doubt concerning the gametic purity of *O. biennis* L.<sup>24</sup>

<sup>20</sup> Die Mutationstheorie. Leipzig. 1900; see 2:599.

<sup>21</sup> Pure seeds of this pure strain I shall be glad to send to any botanist interested in these questions.

<sup>22</sup> Über die Dauer der Mutationsperiode bei *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. 33:387. 1905.

<sup>23</sup> STOMPS, TH. J., Mutation bei *Oenothera biennis* L. Biol. Centralbl. 32:532. 1912.

<sup>24</sup> DAVIS, B. M., Mutations in *Oenothera biennis* L. Amer. Nat. 47:116. 1913; also Parallel mutations in *Oenothera biennis* L. Amer. Nat. 48:498-501. 1914.

From these discoveries it was pretty safe to deduce that the pure *O. biennis* must also be in a state of mutability, and the first thing to do was obviously to make extensive cultures in order to find the pure line mutants. STOMPS cultivated over 900 individuals of the third and fourth generations of a pure line, derived from a rosette collected by him in the sand dunes near Beverwyk, Holland, in 1905.<sup>25</sup> Among these he found one *O. biennis* mut. *nanella*, one *O. biennis* mut. *semigigas*, and also four instances of the pale-yellow variety *O. biennis sulfurea*. The first two he calls *parallel mutations*, since they are analogous to the dwarfs and *semigigas* mutations of *O. Lamarckiana* and arise in the same way and with the same differentiating characters. The experimental origin of *O. biennis sulfurea* by mutation clearly shows that this variety, which is anything but rare in some parts of our sand dunes, may arise in the same way in the wild condition and afterward propagate itself by seeds.

The production of dwarfs from *O. biennis* by mutation has since been repeated more than once in my cultures of hybrids between this species and some of its allies,<sup>26</sup> and a *lata* mutant from *O. biennis* has been reported by GATES and described under the name of *O. biennis* mut. *lata*. Besides *O. biennis*, some allied species also are now known to show the phenomenon of mutation. Among these, an American form of *O. biennis*, which I cultivate under the preliminary name of *O. biennis Chicago*, has been studied more extensively than any other form. I had already found in the neighborhood of Courtney, Miss., in 1904, in a locality called "the bottom," along the shores of the Missouri River, a single specimen with narrow, almost linear leaves. Evidently it constituted a wild mutation from the surrounding type.<sup>27</sup>

Seeds taken from the normal specimens of this locality have since produced in my garden two mutations, which proved, in their progeny, to give constant and uniform strains and which I have cultivated during a series of years under the names of

<sup>25</sup> STOMPS, TH. J., *Parallele Mutationen bei Oenothera biennis* L. Ber. Deutsch. Bot. Gesells. 32:179-188. 1914; also *Parallel mutations in Oenothera biennis* L. Amer. Nat. 48:494-497. 1914.

<sup>26</sup> *Gruppenweise Artbildung*. pp. 300-301. Berlin, 1913.

<sup>27</sup> *Op. cit.* p. 304.

*O. salicastrum* and *O. salicifolia*.<sup>28</sup> The first plants are as high as *O. biennis* Chicago, attaining 2 and sometimes (1914) almost 3 meters in height. They differ mainly in having narrower leaves. The *salicifolia*, on the contrary, is different from its parent species in almost all respects, being richly branched and rarely attaining one meter in height. It has almost linear leaves of a special blotted green, small erect flowers and long thin fruits. Analogous mutations have from time to time been observed in hybrid cultures of *O. biennis* Chicago.

Under the name of metaclinous hybrids I have described the curious phenomenon that heterogamous species from time to time produce among their hybrids from one cross, in one or a very few specimens, the type which is ordinarily that of the reciprocal hybrid.<sup>29</sup> For instance, the cross *O. biennis* Chicago  $\times$  *O. Lamarckiana* gives the twin hybrids *densa* and *laxa*, while *O. Lamarckiana*  $\times$  *O. biennis* Chicago produces the twins *O. laeta* and *O. velutina*. Now among the first hybrid cultures sometimes a *velutina*, and more rarely a *laeta*, arises, and among the latter sometimes a *laxa*. Evidently some latent mutation, on the part of *O. biennis* Chicago, must be responsible for the production of these aberrant types. Analogous metaclinous hybrids have been described for *O. atrovirens* Bartl.<sup>30</sup>

Narrow-leaved mutations have also been seen in cultures of *O. muricata*, and of late (1914) in those of *O. suaveolens* Desf.<sup>31</sup> Moreover, *O. grandiflora*, collected by Mr. BARTLETT and myself at Castleberry in Alabama, throws off aberrant forms, one of which has broader and the other almost linear leaves.<sup>32</sup>

<sup>28</sup> For descriptions and figures see Gruppenweise Artbildung. pp. 304-307.

<sup>29</sup> *Op. cit.* p. 308.

<sup>30</sup> This is the species described in my book Gruppenweise Artbildung under the name of *O. cruciata*. For its metaclinous hybrids see pp. 309-310.

<sup>31</sup> For the different varieties and mutations of *O. muricata* see also GATES, R. R., A contribution to the knowledge of the mutating Oenotheras. Trans. Linn. Soc. II. Bot. 8:1-66. pls. 1-6. 1912.

<sup>32</sup> For *O. grandiflora* see GATES, *op. cit.* p. 38. If the three types of *O. grandiflora*, observed in my garden, occur also at Dixie Landing, Alabama, and have crossed, each of them, with *O. Tracyi*, and have perhaps produced twin hybrids and unlike reciprocals, this might explain the large number of forms observed on that spot; see Science 38:600. 1912.

Lastly, mutations have been observed by H. H. BARTLETT<sup>33</sup> to arise in *O. stenomeres*, a new species of Montgomery, Maryland. In the fourth generation of a pure strain, embracing 106 individuals, he found three aberrant types. One was a self-sterile plant, the second had thick buds and short thick fruits, and the third was a stout and very hairy individual with densely hairy petals, which justify its new name *O. stenomeres* mut. *lasiopetala*. Hairy petals constitute quite a new discontinuous variation among the evening primroses, since all individuals of *O. stenomeres*, as well as the allied species now being studied in this respect, have petals which are glabrous, except under microscopic examination.

From this list we see that at least seven species, besides *O. Lamarckiana*, are now known to be in a condition of mutability, namely *O. biennis* L., *O. biennis* Chicago, *O. muricata* L., *O. atrovirens* Bartl., *O. suaveolens* Desf., *O. grandiflora* Ait.,<sup>34</sup> and *O. stenomeres* Bartl. Probably more or less numerous allied forms will prove to be in the same condition as soon as they are tried on a sufficiently large scale. Therefore, this mutability can no longer be explained on the ground of observed or supposed characters of *O. Lamarckiana* which would distinguish this species from the other types of the group *Onagra*.

*O. biennis* L., the European type of the species, which is growing wild and in large quantities in the sand dunes of Holland, where it had already been observed and collected by LINNAEUS, is, next to *O. Lamarckiana*, the most suitable for researches concerning mutability. DAVIS says, "No wild species of evening primrose has been so long under experimental and field observation or is better known to the workers with *Oenotheras* than this plant. The species has proven uniform to a remarkable degree, and it would be difficult to find a type of *Oenothera* so free from suspicion of gametic purity. The species appears to have been in Holland since pre-Linnean days, and is therefore very old. As material

<sup>33</sup> BARTLETT, H. H., An account of the cruciate-flowered *Oenotheras* of the subgenus *Onagra*. Amer. Jour. Bot. 1:226-243. pls. 19-21. 1914; see p. 236.

<sup>34</sup> Concerning the specific difference of the two last named forms, which have often been considered as synonyms, see *L'Oenothera grandiflora* de l'herbier de Lamarck, Travaux de biologie végétale dédiés à GASTON BONNIER, Rev. Gén. Bot. 25<sup>2</sup>:151-166. fig. 1. 1914.

for experimental studies on mutation, the Dutch *biennis* seems to the writer the best of all *Oenotheras* so far brought into the experimental garden.<sup>35</sup>

In order to determine the coefficient of mutation for *O. biennis* L., I have made a culture of about 8500 individuals, all of which have been studied from their germination to the period of flowering and of fruiting. In the interest of subsequent cultures they have been pulled out before ripening their seeds, with the exception of a sufficient number of their mutants, which were cultivated with some of the true individuals in another garden.

The seeds for this culture were taken from the pure line pedigree plants of STOMPS, which were derived from a single rosette of radical leaves collected by him in 1905 in our sand dunes near Wyk aan Zee.<sup>36</sup> In this part of our country, no other species of *Oenothera* are growing and no intermingling of forms has to be feared. From seed of this plant, self-pollinated, a second generation was grown in 1910 and a third generation in 1912. Self-pollinated individuals of these two generations gave the seed for the cultures of STOMPS in 1913 and for mine in 1914. These latter came from three and four parent plants, the descendants of which numbered respectively 5500 and 3000. Of course I sowed almost all the available seed, and their culture just covered the field at my disposal outside of my experimental garden (about 600 square meters). Thus all my plants belonged to the same pure line as those of STOMPS, and the individuals which supplied the seeds had been cultivated under the most favorable conditions obtainable.

The seeds were sown in January, the seedlings transplanted into wooden boxes in March, and brought on the field in the middle of April. This early sowing and transplanting is with us the most effective means of making the plants annual, and in my whole culture less than a dozen individuals failed to flower.

It was possible, this time, to pick out the dwarfs from the wooden boxes before the transplanting into the field. By this means a second change of place was avoided, and the dwarfs could

<sup>35</sup> DAVIS, B. M., Parallel mutations in *Oenothera biennis* L. Amer. Nat. 48:499-1914.

<sup>36</sup> STOMPS, TH. J., Parallele Mutationen bei *Oenothera biennis* L. Ber. Deutsch. Bot. Gesells. 32:179-188. 1914.



be cultivated together on a bed of my experiment garden, which enabled me to inspect them almost every day during their development and through the whole summer. The characters which distinguish the dwarfs in the stage of young rosettes, with leaves a few centimeters in length, were discovered in the following way.

The self-pollinated flowers of the dwarf specimen of STOMPS in 1913 had set no good seeds, but flowers pollinated from pure *biennis* had produced some fruits. Now my *O. Lamarckiana* mut. *nanella*, when crossed with *O. biennis*, yields only, or almost only, dwarfs. Therefore, the expectation was justified that such might also be the result of the cross *O. biennis* mut. *nanella* × *O. biennis*. Seeds from this cross had been sown about the same time; they yielded 108 seedlings, all of which have been planted out and have flowered. They were dwarfs without exception, reached in September a height of 40–45 cm. only, were richly branched, and had all the marks of *O. biennis* combined with the dwarfish stature and the liability to the same bacterial disease as is shown by the dwarfs of *O. Lamarckiana*. The young rosettes of these crossed *biennis* dwarfs clearly differed from the rosettes of the pure *biennis*. After the three or four first leaves with long petioles, there followed a group of leaves with smaller stalks and some sessile ones, thereby rendering the whole rosette far more compact than the corresponding ones of the pure *biennis*. With this character as a criterion, I isolated from my pure line boxes 8 individuals. One of them proved afterward to be a mistake; it was a pure *biennis*. Seven were dwarfs and have flowered; they were, in all external respects, like the crossed dwarfs of the control culture. Among the 8500 remaining plants I discovered later, in the field, only one dwarf. This shows that the characters were sufficiently reliable. All in all, I had 8 dwarfs in 8500 plants, making about 0.1 per cent. They occurred among the progeny of one of the self-pollinated mothers in the second generation (3 dwarfs), and of three of the parents in the third generation (5 dwarfs). Some of them have set good fruits after self-fertilization.

One of the most interesting and useful features of *O. biennis* L. is its propensity to make lateral rosettes from the base of the flowering stem. It is possible to isolate these rosettes and to have

them grow separately. The experiment succeeds easily if the rosettes have produced one or two roots of their own, however young and slender these may be. *O. biennis nanella* shows the same character, and in August I succeeded in isolating from my 8 pure line dwarfs 8 rosettes, all of which have since developed into healthy young plants with some long and narrow leaves, followed by almost sessile ones, quite different from the rosettes of normal *O. biennis*.

Moreover, two *nanella* mutants occurred in the cultures of *O. biennis sulfurea* which I shall have to describe later. These cultures were grown from self-pollinated seeds of the four *sulfurea* mutants of STOMPS (1913), and embraced over 1000 flowering individuals, the flowers of which were pale yellow without exception. Two of these plants proved to be dwarfs and were transplanted into my experimental garden. Both of them have flowered with pale flowers, have been self-pollinated, and yielded a sufficient harvest of seeds. The coefficient of mutation in this race was therefore 0.2 per cent, which does not differ essentially from the first instance (0.1 per cent). These dwarfs are the founders of a new race, *O. biennis sulfurea nanella*, which I propose to cultivate next year. Its pedigree name would be *O. biennis* mut. (1913) *sulfurea* mut. (1914) *nanella*. It is a double mutant, such as are quite common in horticulture, and shows the way in which wild species would have to be analyzed.

I used the pollen of the *O. biennis nanella* of STOMPS, in 1913, for two crosses, which may be briefly mentioned here. In the first place, I fertilized castrated flowers of the pure line of *O. biennis*. The pollen was not abundant, and I got only 15 good seeds, all of which have germinated and become stout flowering plants. They differed from normal *O. biennis* in no respect and at no moment during their development. Their self-pollinated seeds will have to be sown next year. In the second place, I pollinated *O. Lamarckiana* with the pollen of *O. biennis nanella*. From this cross I had a culture of 55 individuals, all of which have flowered. One of them proved to be a *lata* mutant, having besides the *lata* marks the same characters as its sisters. These were all alike and in no way different from the ordinary and well known type of *O. Lamarck-*

*iana* × *biennis*, which, moreover, flowered at the same time on other plots of my garden. A number of these plants have been self-pollinated. Thus we see that the pollen of *O. biennis nanella* gives in these cases exactly the same forms as that of pure *O. biennis*, at least so far as the first generation is concerned.

The specimen of *O. biennis semigigas* of the cultures of STOMPS had only matured seed in the capsules which had been pollinated by pure *O. biennis*, without being castrated. From these seeds two types arose, neither of which was a *semigigas*. All in all, there were 19 plants, belonging to two forms, besides a mutant. This last was a dwarf, which, however, has not flowered. Of the remainder, ten individuals were pure *biennis* during their whole life and in all their marks. They had the normal number of chromosomes, namely 14, and gave a normal harvest of seeds. The others, 8 in number, were different from these in almost all respects, though but slightly. The color of their foliage was a whitish green, the leaves more flat, and with white veins. The spikes were more elongated, the flower buds more slender, the flowers small and erect, the fruits thin and cylindrical and relatively poor in seeds. These plants had 15 chromosomes, like the *O. Lamarckiana lata* studied recently by GATES and Miss THOMAS.<sup>37</sup> But they had none of the characters of a *lata*, showing thereby that the number of chromosomes, even if differing from the type, does not necessarily run parallel with the external features.

Further studies will have to show why one-half of the progeny of this cross came true to the characters of the pollen parent, while the other half constituted a new and uniform type, differing from all the mutations and hybrids hitherto studied in my experiment garden; and especially why the characters of the mother of the cross should be wholly absent in its progeny.

The first result of this state of affairs has been that the characters which the *semigigas* mutants might show in early youth remained unknown, and that it has not been possible to point them out before the time of flowering. In July, all the spikes

<sup>37</sup> GATES, R. R., and THOMAS, N., A cytological study of *Oenothera mut. lata* and *O. mut. semilata* in relation to mutation. Quar. Jour. Micr. Sci. 59:523. 1914.

were carefully mustered and four specimens of the *semigigas* type were discovered. This makes a proportion of 4 to 8500, or about 0.05 per cent, showing the *semigigas* mutants to be only half as frequent as the *nanella*. On later inspections no additional cases were observed, and likewise intermediate or doubtful instances were absent. The four plants were exactly alike, save that three were very vigorous, and one, grown in a shady part of the garden, was very weak. The chromosomes were counted in the first three instances and found to be 21, as in the corresponding mutant of STOMPS.

My four mutants were easily discovered by their broad conical flower buds and their elongated spikes, which strongly contrasted with the dense spikes of the surrounding *biennis*. They reached the same height as these, the lowest flower being 90 cm. above the soil, and the total height about 1.5 meters. The leaves had the same form as those of *biennis*, but were a darker green and slightly more pubescent. The pollen consisted of 3- and 4-cornered grains, both of which types seemed fertile only for about a quarter. Artificial self-fertilization, however, had no result, and on the stigmas of *O. biennis*, *O. gigas*, and *O. Lamarckiana* the effect of the pollen was very slight, inducing some swelling of the ovaries but no good seeds or almost none. Inversely, I have tried to fertilize the flowers with the pollen of the three species named, but got a good result only in the case of *O. biennis*. Numerous good capsules with a sufficient supply of apparently good but in reality empty seeds have been obtained by leaving the flowers free to the agency of insects in the midst of the thousands of their flowering sisters, while in the same garden no other *Oenotheras* were grown.

The three vigorous specimens of the mutant produced some lateral rosettes at the base of their stem, even as we have seen in the case of the parent species and the dwarf variety. These rosettes were isolated and planted in pots in the beginning of August; four of them were very vigorous, but the other one rather weak. They have thrown off lateral rosettes themselves, and the stems repeated the production in two instances. It is proposed to try to bring these plants through the winter and repeat with them the culture and the experiments of this year. After a month, their

leaves reached 15 cm. and more in length and were clearly distinct from the normal type of *O. biennis*, being much broader and a darker green.

Of the four *semigigas* mutants, two arose from the seeds of the same parent which yielded the *semigigas* of STOMPS in 1913. All three belonged to the third generation of the pedigree. The two others were derived from two different parents of this same generation and therefore belonged to the fourth. The reason why three of the five came from the same lot of seed was probably no other than that the harvest of this plant had been the largest. More than one-third of my whole culture (3200 plants) were children of this mother.

No *gigas* with 28 chromosomes and fertile pollen occurred in my culture. With a chance of one sexual cell mutated into *O. gigas* in every 2000, the expectation for the copulation of two such cells is evidently only one in every 4,000,000. This would require a garden of more than five or six acres (two hectares) and the corresponding cost of labor. Perhaps some American institution is able to carry out the experiment. It may be reduced very essentially by a previous study of the marks of the young rosettes of *O. biennis semigigas*, so as to be able to plant out almost only these, hoping to find the *gigas* among them; or by studying the external influences which may increase the degree of mutability of the parents in the desired direction.

*Sulfurea* mutants have been far less rare. This was to be expected from the fact that STOMPS had 4 of them among 920 plants. From the parent type they differ only in the color of their petals, which is a very pale yellow. It is so pale that collectors, who see the variety in our sand dunes, often call the petals white. In the cultures they are easily seen as soon as the flowers open, especially in the evening. I found 27 of them among my 8500 plants, making a percentage of 0.3 per cent. They occurred in the progeny of all the 7 parents of my stock, 13 in the third, and 14 in the fourth generation. There were 6 parents, whose progeny contained 0.1–0.3 per cent, and one with 0.7 per cent (of the fourth generation). It is possible that this last parent had been more favored by external conditions than the three others of the

same group, although it grew among them and did not show any higher degree of vigor.

The fact that *sulfurea* mutants were observed in the progeny of every one of the 7 parents of my culture directly proves this line of mutability to be hereditary in the whole family derived from the 1905 rosette from Wyk aan Zee. In combination with the sporadic occurrence of the pale-colored variety in our sand dunes, we may further infer that this mutability is hereditary in the whole stock of our country, and probably also in the whole species, since *sulfurea* plants have been found from the time of TOURNEFORT in France and other European countries.

From the mutants constant races may be derived. I sowed the self-pollinated seeds of the four mutants of STOMPS, and cultivated 205, 225, 271, and 358 seedlings, altogether 1059 plants, all of which have flowered and produced only pale-yellow petals, making a very striking impression of constancy.<sup>38</sup> When crossed with the pure species, the *sulfurea* strains give uniform hybrids which are patroclinous. Those of *O. biennis* × *sulfurea* have the pale flowers, those of *O. biennis sulfurea* × *biennis* show the same bright yellow as the parent species.<sup>39</sup>

### On experimental germination of seeds

Of the seeds of *Oenothera Lamarckiana* ordinarily only about one-third produce seedlings, and this proportion is highly variable, depending mainly on the conditions of cultivation of the parent plant. Among the remaining seeds some contain a normal embryo, others a more or less completely decayed one, while still others are empty. The last have been thoroughly studied by RENNERT, who found that they have been fertilized as well as the normal seeds and those with decayed embryos. Between these normal and externally normal seeds are seen the numerous rudimentary ovules which have not been fertilized, and have not essentially increased their size after the fertilization of the others. These rudimentary seeds have been described by GEERTS, as referred to above.

<sup>38</sup> Self-pollinated seeds of this second generation of *O. biennis sulfurea* are available for exchange in return for other races of mutating primroses.

<sup>39</sup> Gruppenweise Artbildung. p. 298.

In the empty seeds the embryo develops only a little, just enough to stimulate the seed coats to an almost normal development, in size as well as in structure. For the most part these empty seeds are a little smaller and especially a little less broad than the others, and can therefore easily be picked out of a sample. But quite a good many are externally exactly like good seeds and cannot be distinguished from them without being opened. RENNER states that about one-half of the seeds are in this empty condition.

By means of a hard steel needle with a curved tip it is easy to make the seeds burst, especially after a thorough wetting. The seeds which contain a healthy embryo will discharge it; the unhealthy seeds will protrude a slightly brownish pulp; and the empty seeds show the lack of contents, except a thin layer of endosperm in the embryo sack. The various groups may be counted out in this way, but the limits between the originally empty seeds and those which have become more or less empty by an early decaying of their germs are not sharp and often dependent upon the health conditions of the seed-bearing individual.

Among the seeds with a normal and healthy embryo some will germinate during the first days after sowing, especially if the temperature is a favorable one. Others will follow sooner or later, some after weeks or months, while still others may remain dormant for years. It is not an uncommon case that the proportion of the rapidly germinating seeds is a very small one, and in this case a large quantity of seed is necessary to secure a small number of seedlings. Moreover, in those cases where the seeds do not produce a uniform progeny, but a mixture, as, for example, with twin hybrids or in hybrid splitting, the possibility cannot be denied that the numerical proportion of the components of the mixture may be different for the rapidly germinating seeds as compared with the others. In other words, percentage figures may be influenced to some degree by the occurrence of a more or less considerable proportion of dormant seeds.

In order to ascertain the value of this objection, I have made from time to time cultures in which the rapidly germinated seedlings were planted out separately from the slower ones. As a

matter of fact, I have not found as yet any essential differences between the two groups; but the doubt remained that such might still be discovered if it were possible to bring to germination all, or almost all, the slow seeds of a given sample. For a number of years I have tried various means to reach this end, but only of late have I succeeded.

It is a well known fact that many kinds of hard seeds may be induced to germinate by means of filing. Filing machines, especially for the smaller leguminous seeds, are now often used in agricultural practice, the best known one being the Swedish type, constructed by HJALMAR NILSSON, the Director of the Swedish Agricultural Experiment Station at Svalöf. It files the seeds in a continuous current by throwing them against a rapidly revolving filing disk. Unfortunately, in the seeds of the evening primroses, the hard layer is not the external tissue, but that of the inner integument. The outer coat thus prevents the filing, and experiments which Professor NILSSON has had the kindness to make for me with his apparatus did not give the desired result.

In the soil the water is imbibed into the seeds through microscopic and very narrow slits in the hard layer. It is assumed that these slits are filled with air which, in the narrower ones, is a powerful obstacle against the penetration of the water. So long as this only reaches the cuticularized parts of the walls of the slits, no moisture can reach the embryo and this remains dormant. The question, therefore, is to compel the water to penetrate into the deeper parts of the slits so as to reach the spots which can be moistened.

In order to solve this difficulty, I have tried pushing the water into the slits under a high pressure. A compression of the surrounding air to 6-8 atmospheres has proved to be sufficient to induce all or almost all the healthy seeds to germinate in a few days. The apparatus used is a combination of an autoclave with an air-pump such as is used for automobiles, and the model known as the Michelin pump seems to be the easiest and cheapest available one, while any autoclave, as, for example, an ordinary steam sterilizer, will answer the purpose. Mine has 20 cm. inside diameter, and can be filled to 8 atmospheres in about five minutes.



Before compressing the air in the seeds, these are thoroughly soaked with water. Ordinarily they are exposed in small tubes, half filled with water, to a temperature of about 30° C. during one night. In the autoclave they remain from one to three days, at the temperature of the room. My apparatus can accommodate over 100 tubes at a time, each containing a different sample. After leaving the reservoir, the seeds may be sown in seed pans for cultivation or may be left to germinate in the same tubes, if it is only intended to determine the proportion of seedlings produced. In order to do this the water is poured off through a small sieve, the tube is closed by means of a cork, and the seeds are distributed along the upper inner side of the tube, this lying horizontally. In this way they get exactly the required amount of water and of air for a vigorous germination.

I will now give some figures to show the effect of this pumping in of air into the previously soaked seeds. After pumping, the degree of germination was determined by leaving the tubes in a stove at 30° C. and counting the seedlings in samples of about 200 seeds each. Out of 18 capsules from self-fertilized flowers of a spike of *O. Lamarckiana*, 3400 seeds were counted, a separate germinating tube being used for the contents of each fruit. Of these seeds, 15 per cent germinated during the first two days and only 3 per cent during the two following days, showing the normal germination power to be almost exhausted. Then the seeds remained three days in water under a pressure of 8 atmospheres, after which they were brought back to the stove. The next two days produced 22 per cent seedlings, and the four following ones added only 1 per cent to this number. Then the remaining seeds were tried with a needle. Only about 5 per cent contained embryos, half of which at least were evidently in a decaying condition.

The total of germs was 46 per cent, leaving 54 per cent for those with an undeveloped germ. From these figures we see that the production of seedlings from a sample of seeds may be more than doubled by the pumping method, while all or almost all the healthy germs may be made to germinate. Numerous similar instances could be added.

A sample of seeds of *O. biennis*, taken from a late flowering individual, produced only 2 per cent of seedlings in the first two days, while a control sample, after having been exposed in water to a pressure of 6 atmospheres, produced at once 80 per cent of seedlings. In the same way for *O. suaveolens*, the percentage was increased from 3 to 14 per cent, for *O. muricata* from 12 to 80 per cent, and for *O. Cockerelli*, a species which is often very slow in germinating, from 2 to 72 per cent.

It is not improbable that in *O. Lamarckiana* the hard seeds may contain more mutants than the easily germinating ones, which have thus far been studied. It seems even possible that they may conceal some new, as yet unobserved, types of mutations. The new method enables us to bring almost all the germs to germination, as well as to separate the seedlings of the different groups.

Before concluding, I may be allowed to recommend this method for the study of various other kinds of seeds also.

### Summary

1. In a culture of 8500 specimens of pure line *Oenothera biennis* L., 8 mut. *nanella*, 4 mut. *semigigas*, and 27 mut. *sulfurea* arose, giving the percentages of about 0.1 per cent, 0.05 per cent, and 0.3 per cent. In cultures of *O. Lamarckiana* the corresponding numbers are for *O. nanella* 1-2 per cent, for *O. semigigas* 0.3 per cent (Gruppenweise Artbildung. p. 329), while no color mutations have been observed as yet. With the origin of *O. Lamarckiana* the mutability for dwarfs, therefore, must have increased at least tenfold, and for *gigas* types about sixfold. The material cause for this improvement is in all probability the same as or closely connected with the cause of the largely increased number of mutative forms which are known to start from *O. Lamarckiana*.

2. From the cross *O. biennis* mut. *nanella* × *O. biennis* only dwarfs of a uniform type arose (108 Ex). *O. biennis* × *O. biennis* mut. *nanella* was in the first generation exactly like pure *biennis*; *O. Lamarckiana* × *O. biennis* mut. *nanella* exactly like *O. Lamarckiana* × *biennis*.

*O. biennis semigigas* is self-sterile, but when pollinated by *O. biennis* gives for one-half pure *biennis* with 14 chromosomes, and for the other half a new, slender type with 15 chromosomes.

*O. biennis* mut. *sulfurea* easily yields constant races of a uniform sulphur color.

3. The question whether there is any causal relation between partial sterility of the sexual cells, hybridism, and mutability has to be studied in all those instances in which mutations are known to occur or to have occurred. In some of these cases, at least, the conditions are far more simple than for the evening primroses, as for example in *Capsella Bursa-pastoris*.

4. The mutative condition of *O. biennis* may be ascribed to some "germinal disturbance" of its hereditary qualities. Or, if we replace this vague and meaningless expression by a sharp hypothesis, we may assume as its cause the presence of one or more pangens in a labile position. The transition from *biennis* to *Lamarckiana* would then require the addition of one or more pangens in the same state, in order to explain the higher percentage of mutants and the larger number of their different forms. The presence of such labile pangens seems well proven by the results of numerous crosses.

The contention, however, that the transition of "undisturbed germinal material into a state of disturbance," or of one or more pangens from the stabile into the labile condition, may be induced by external influences in pure species, has not as yet found general acceptance. Some authors believe that crosses between different types are required to secure this effect. At this moment, it seems difficult to give experimental evidence for or against this view. Until this is reached, we must rely upon comparative studies in order to answer the main question whether or not the observed mutations in the evening primroses are analogous to those by which the mutation theory explains the evolution of the animal and vegetable kingdoms.

5. The mutants of *O. Lamarckiana* all agree with that species in certain characters, and not one of them shows any indication of a reversion toward any of the allied wild types. If the mutability was an effect of crossing, some marks, at least, of the other parent would be expected to reappear.

Besides this consideration, the available evidence lies in the fact that the derivatives of *O. Lamarckiana*, originated in my garden, differ from one another in marks, which are, although not

identical, strictly analogous to those which differentiate the wild species of the whole group. In some cases the differences are even larger. Those between the wild species are often very small and limited to certain life periods, leaving the species quite alike during the remainder of their development. No arguments have as yet been adduced to doubt the fundamental identity of the two groups of characters.

6. The phenomenon of mutability, observed in *O. Lamarckiana*, *O. biennis*, and allied forms, is therefore to be considered as a simple continuance of the supposed mutability which presided at the origin of the wild species of the evening primroses.

7. The seeds of the evening primroses are often very slow in germinating, leaving sometimes one half or more of the healthy germs in a dormant condition. This difficulty in the study of mutation percentages, etc., may be overcome by pressing the water into them. A pressure of 6–8 atmospheres during 1–3 days is ordinarily sufficient to stimulate all or almost all the good germs to a rapid germination.

The microscopic preparations and the counts of chromosomes, referred to in this article, have been made for me by my assistant Mr. C. VAN OVEREEM, to whom I wish to give my sincere thanks for his cooperation.

AMSTERDAM, HOLLAND